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Biochimica et Biophysica Acta 1772 (2007) 195–204
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbadis

Review

Myotonic dystrophy: Emerging mechanisms for DM1 and DM2

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Received 3 April 2006; received in revised form 26 May 2006; accepted 26 May 2006
Available online 20 June 2006

Abstract

Myotonic dystrophy (DM) is a complex multisystemic disorder linked to two different genetic loci. Myotonic dystrophy type 1 (DM1) is caused by an expansion of a CTG repeat located in the 3′ untranslated region (UTR) of *DMPK* (myotonic dystrophy protein kinase) on chromosome 19q13.3. Myotonic dystrophy type 2 (DM2) is caused by an unstable CCTG repeat in intron 1 of *ZNF9* (zinc finger protein 9) on chromosome 3q21. Therefore, both DM1 and DM2 are caused by a repeat expansion in a region transcribed into RNA but not translated into protein. The discovery that these two distinct mutations cause largely similar clinical syndromes put emphasis on the molecular properties they have in common, namely, RNA transcripts containing expanded, non-translated repeats. The mutant RNA transcripts of DM1 and DM2 aberrantly affect the splicing of the same target RNAs, such as chloride channel 1 (ClC-1) and insulin receptor (INSR), resulting in their shared myotonia and insulin resistance. Whether the entire disease pathology of DM1 and DM2 is caused by interference in RNA processing remains to be seen. This review focuses on the molecular significance of the similarities and differences between DM1 and DM2 in understanding the disease pathology of myotonic dystrophy.

Keywords: Myotonic dystrophy; Repeat expansion; DMPK; ZNF9; MBNL; CUG-BP

1. Introduction

1.1. Overview of repeat expansion disorders

Myotonic dystrophy is currently one of over 30 neurological disorders that can attribute their pathogenesis to extensions of tandem repeats above a critical size. Yet the theories as to how large repeat arrays can cause such highly variable and multifaceted diseases are numerous and unresolved [1]. In general, the repeats become meiotically unstable above a certain pathogenic size threshold, and often demonstrate tissue-specific somatic mosaicism of increasing repeat lengths over time. Many of the disorders also show a clear propensity for inheritance of a more severe disease phenotype with increasing penetrance than previous generations, termed anticipation [2,3]. Most of the expansion disorders exhibit a delayed onset form of their diseases, indicating that they may share mechanisms that postpone clinical expression until later in life. The population maintenance of these disorders marked by low reproductive fitness also indicates that there might be a common mechanism of generating new expansions from an existing pool of normal size repeats [4,5]. Lastly, nuclear inclusions typically characterize repeat expansion disorders, but they begin to differ by whether these aberrant aggregations are derived from mutant protein or RNA transcripts.

Depending on where the unstable repeats are located within the gene, the repeat expansion disorders can be classified as having coding or noncoding mutations. Coincidentally, in 1991, the first two triplet repeat expansion disorders discovered revealed examples from both coding and noncoding categories — the fragile X syndrome was linked to unstable CGG repeats in the noncoding 5′UTR of *FMR1* [6–8], whereas spinobulbar muscular atrophy was associated with unstable CAG repeats in the coding region of *AR* [9]. DM1 followed in 1992 as the third trinucleotide repeat expansion disorder discovered and was mapped to CTG repeats in the noncoding 3′UTR of *DMPK* [10–13]. Coding region ex-

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doi:10.1016/j.bbadis.2006.05.013

pansion disorders such as Huntington's disease (HD), dentatorubralpallidoluysian atrophy (DRPLA), and spinocerebellar ataxia (SCA) 1, 2, 3, 6, 7, and 17, are comprised of CAG repeats that become translated into polyglutamine tracts. Polyglutamine extensions from 40 to 100 residues become toxic to cells of the central nervous system [14]. Noncoding triplet repeat expansion diseases such as in SCA8, fragile X syndrome, and Friedreich ataxia encode tandem CTG, CGG or GAA repeat units, respectively [15,16]. Diseases caused by noncoding repeats exhibit a higher range of pathogenic repeat lengths, often numbering in the hundreds or thousands of repeats.

Unlike the gain-of-function mutations of the altered protein products from the polyglutamine expansion disorders, the noncoding expansion disorders appear to result from either a loss-of-function by transcriptional repression or a gain-of-function mediated by the mutant RNA transcripts. For example, hypermethylation of the expanded CGG repeat region in the 5′ UTR of *FMR1* silences expression of the gene [17–19]. In agreement with the loss-of-function model, mice deficient in *Fmr1* display a similar reduction in cognitive abilities as humans with fragile X syndrome [20]. In contrast, both DM1 and SCA8 express RNA with untranslated CTG repeats in the 3′ UTR domains of their respective genes. The fact that SCA8 is predicted to be a noncoding RNA highlights how protein function of the mutant genes may not be primarily involved in mediating these particular disorders. It is, however, studies on myotonic dystrophy that has first unveiled the critical role of RNA in contributing to disease phenotype.

1.2. Myotonic dystrophy

Myotonic dystrophy (Steinert's disease, 1909) is the most prevalent form of muscular dystrophy with a frequency of 1 in 8000 individuals worldwide [21]. Affected individuals express highly heterogeneous, multisystemic symptoms including myotonia (muscle hyperexcitability), progressive muscle weakness and wasting, cataract development, testicular atrophy, and cardiac conduction defects [22,23]. It has an autosomal dominant mode of inheritance and disease severity generally correlates with repeat length. The first insight into the molecular mechanism of this disorder came from its linkage to chromosome 19q13.3 and subsequent identification of an expanded CTG repeat in the 3′ UTR of *DMPK* in patients with DM1. Wildtype individuals have 5–38 CTG repeats at this locus, whereas individuals with DM1 have repeats in the hundreds to thousands [24].

Once the genetic mutation for DM1 was identified, it became clear that there was a subset of DM patients who did not have CTG expansions at the DM1 locus, but did share significant clinical aspects of the disease, including an autosomal dominant mode of inheritance, myopathy, myotonia, cataracts, and cardiac disturbances [25,26]. The early pattern of muscle weakness was proximal in these patients, often involving hip flexors and extensors, which initially distinguished them from DM1 patients who displayed an early pattern of distal muscle weakness, such as those of the face, neck and fingers [27,28]. Hence, proximal myotonic myopathy (PROMM) was the initial term for those who did not have DM1 [26]. Manual strength tests, however, showed that PROMM patients also shared early patterns of muscle weakness in the distal neck and finger flexors, but in a less prominent manner than DM1 [27,29]. As the disease progresses, the muscle degeneration pattern may overlap and reach proximal and distal muscles for DM1 and DM2, respectively [30]. Almost a decade after the discovery of unstable CTG repeats in 3′ UTR of *DMPK*, Liquori et al. revealed that a CCTG expansion in intron 1 of *ZNF9* on chromosome 3q21 is associated with individuals who were diagnosed with having PROMM/DM2 [31]. This new locus associated with the myotonic dystrophy disorder has been designated as DM2. The frequency of DM2 is uncertain, but most recent reports suggest that its incidence could be as high as DM1 [32].

Both DM1 and DM2 share repeat expansions in noncoding regions of genes (*DMPK* and *ZNF9*) that are expressed in tissues affected by this disease. DMPK, a serine-threonine kinase [3,13], shows no functional similarity to ZNF9, a 7 zinc finger protein thought to bind RNA [33–35]. The fact that both genes were associated by repeat expansions in transcribed but untranslated regions suggested that the mutant RNA might have a significant role in the disease process. However, a key difference between DM1 and DM2 is that only the DM1 locus presents a congenital form of this disorder not shared by DM2 [23,36]. This strongly implies that although the basic features of adult and congenital DM may stem from the transcription of expanded CUG/CCUG repeats, regardless of the encoding gene, additional qualities unique to early onset DM1 may be ascribed to changes at the DM1 locus.

1.3. Clinical features of DM1 and DM2

DM1 and DM2 manifest signs of myotonia, muscle weakness, and early cataract development as the principal traits of myotonic dystrophy [27,30,37]. Changes in chloride conductance from defects in ClC-1 protein function account for the myotonia observed in most symptomatic adults [38,39]. Its accompaniment with progressive muscle degeneration distinguishes this disorder from other forms of clinical myotonias. Although the initial pattern of muscle weakness is noticeably different between DM1 and DM2 (distal vs proximal), the muscle biopsies show a similar histology of central nucleation and increased fiber sizes. Muscle atrophy, as shown by ATPase staining, occurs preferentially in type 1 fibers in DM1 and in type 2 fibers in DM2 [40]. The most treatable symptom of DM is cataract development beginning in the second decade or later and is characterized by multicolored lens opacities on slit-lamp examination. Additional common features include testicular atrophy, frontal balding, insulin insensitivity and hypogammaglobulinemia (reduced IgG and IgM serum levels). Cardiac problems, typified by varied and potentially lethal arrhythmias and occasional signs of cardiomyopathy, are additional examples of the multisystemic nature of this disease [37].

DM2 is considered to present a milder version of this disorder compared to DM1 in most aspects of their related clinical phenotypes although the CCTG expansions can be much larger than DM1 ranging in size from 75 to 11,000 repeats [31] (Fig. 1). In addition, there is no strong evidence for anticipation in DM2 as there is in DM1, in which successive generations inherit increasing disease severity with decreasing age of onset [29]. Most of the clinical features of DM2 appear in adulthood (median age 48 years) [29] as opposed to DM1 that clearly demonstrates adult-onset, childhood-onset and congenital forms with corresponding increasing disease severity and repeat size. The most significant difference between DM1 and DM2 is the presence of the congenital form of DM (hereafter referred to as congenital DM1) that is absent in DM2. Neonatal symptoms of congenital DM1 do not include some of the features characteristic of adult onset DM1 and DM2, such as cataract development, myotonia and myopathy [41]. Instead, congenital DM1 is associated with hypotonia, mental retardation, facial diplegia and a maternal bias in DM transmission [36,42,43]. And rather than progressive muscle degeneration, congenital DM1 exhibits skeletal muscle immaturity that makes this early onset form of DM1 distinct from the adult-onset examples of DM1 and DM2 [44,45]. To a somewhat lesser degree, childhood-onset in DM1 also exhibits cognitive impairment without the maternal bias in transmission seen in congenital DM1 [23,46]. Although there are a few reports of childhood-onset in DM2, they are not usually associated with the developmental disease of the central nervous system as in congenital and childhood-onset DM1. Surviving congenital and childhood-onset DM1 patients eventually manifest the hallmark features of the adult-onset form of the disorder.

The clinical complexity produced by dynamic mutations from two different loci engendered diverse models for the pathobiology of this expansion disorder.

1.4. Model mechanisms for myotonic dystrophy

There are several theories as to the molecular pathophysiology of DM1 [47]. In all, the expanded CTG repeats are proposed to disrupt normal cellular processes at the RNA, protein or chromatin level. Initial research targeted the role of the DMPK protein, especially in light of the fact that several studies showed that cytoplasmic DMPK was reduced in DM1 patients, presumably due to the deleterious effects of the expanded repeats on RNA processing or nucleocytoplasmic transport [48–50]. To test that haploinsufficiency of DMPK may elicit signs of myotonic dystrophy, two groups eliminated its function in mice and demonstrated that mice homozygous for the DMPK deletion developed a late onset mild myopathy [51,52]. Mice both heterozygous and homozygous for the disrupted DMPK gene also displayed cardiac conduction defects [53]. In addition, skeletal muscle cells and cardiac myocytes isolated from heterozygous and homozygous DMPK-deficient mice exhibited abnormalities in Na^+ channel gating and Ca^{2+} cycling akin to

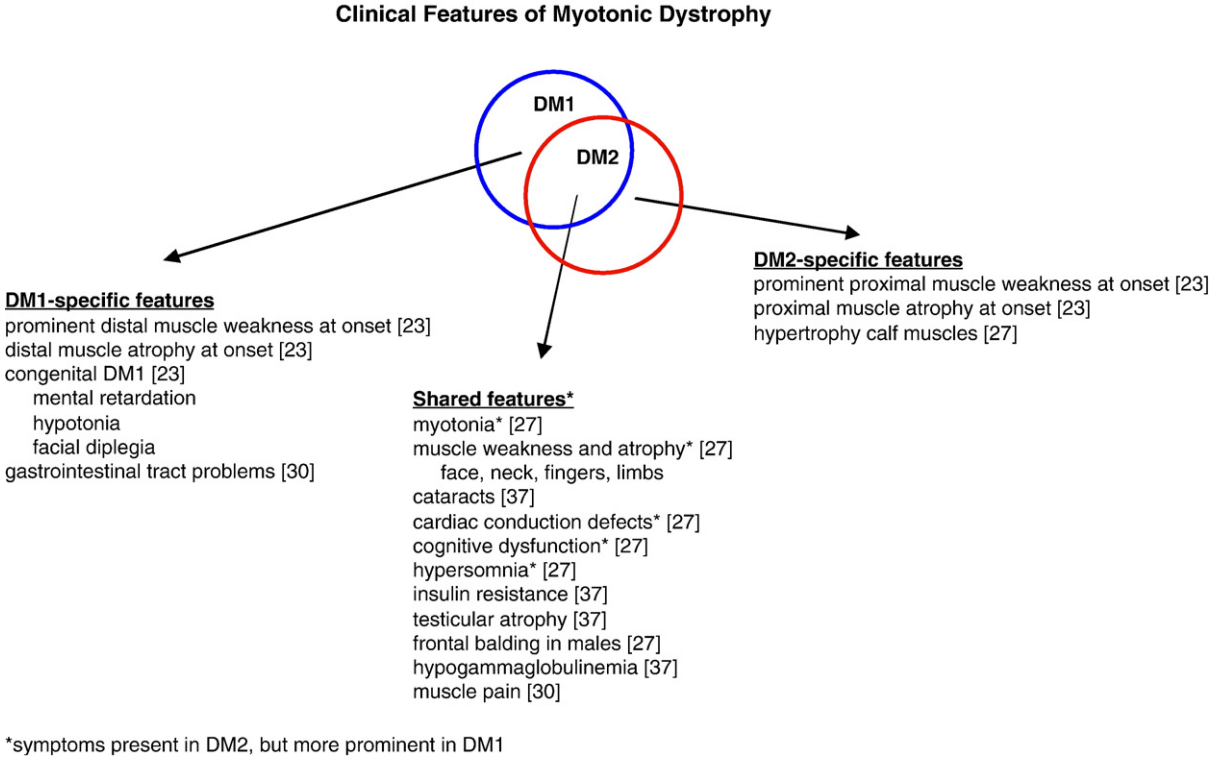

Fig. 1. Clinical features of myotonic dystrophy. Myotonic dystrophy type 1 (DM1) and type 2 (DM2) share several hallmark clinical traits affecting the muscle, eye, heart, brain and endrocrine systems. DM1-specific features include a distal pattern of muscle weakness and atrophy at disease onset, congenital DM1 with associated symptoms of mental retardation, hypotonia and facial diplegia, as well as gastrointestinal tract problems (congenital and late onset) that are notably absent in reports of DM2. Clinical symptoms unique to DM2 include a pronounced proximal distribution of muscle weakness and atrophy at disease onset and hypertrophy of calf muscles.

DM1 patients, which implicated DMPK in muscle weakness and cardiac dysfunction through its involvement in ion homeostasis [54,55]. The mild and partial DM phenotype observed in the DMPK knockout mice also indicated that other models were necessary to fully explain the complex disease phenomenon.

There is strong corroboration for the hypothesis that DM is primarily an RNA-mediated disease (Fig. 2). Initial studies into the intracellular distribution of DMPK transcripts surprisingly revealed that DM1 cells were associated with multiple nuclear foci of mutant DMPK RNA [56]. This visual cue led to the novel theory that the ribonuclear inclusions signify a toxic gain-of-function effect mediated at the RNA level [57,58]. In search of proteins that bind to the anomalous triplet repeat expansion, in vitro studies identified CUG-binding protein (CUG-BP1), which belongs to the CELF (CUG-BP1 and ETR-3-like factors) family of proteins [59–61]. A major insight into the cellular process that might be impaired in myotonic dystrophy came

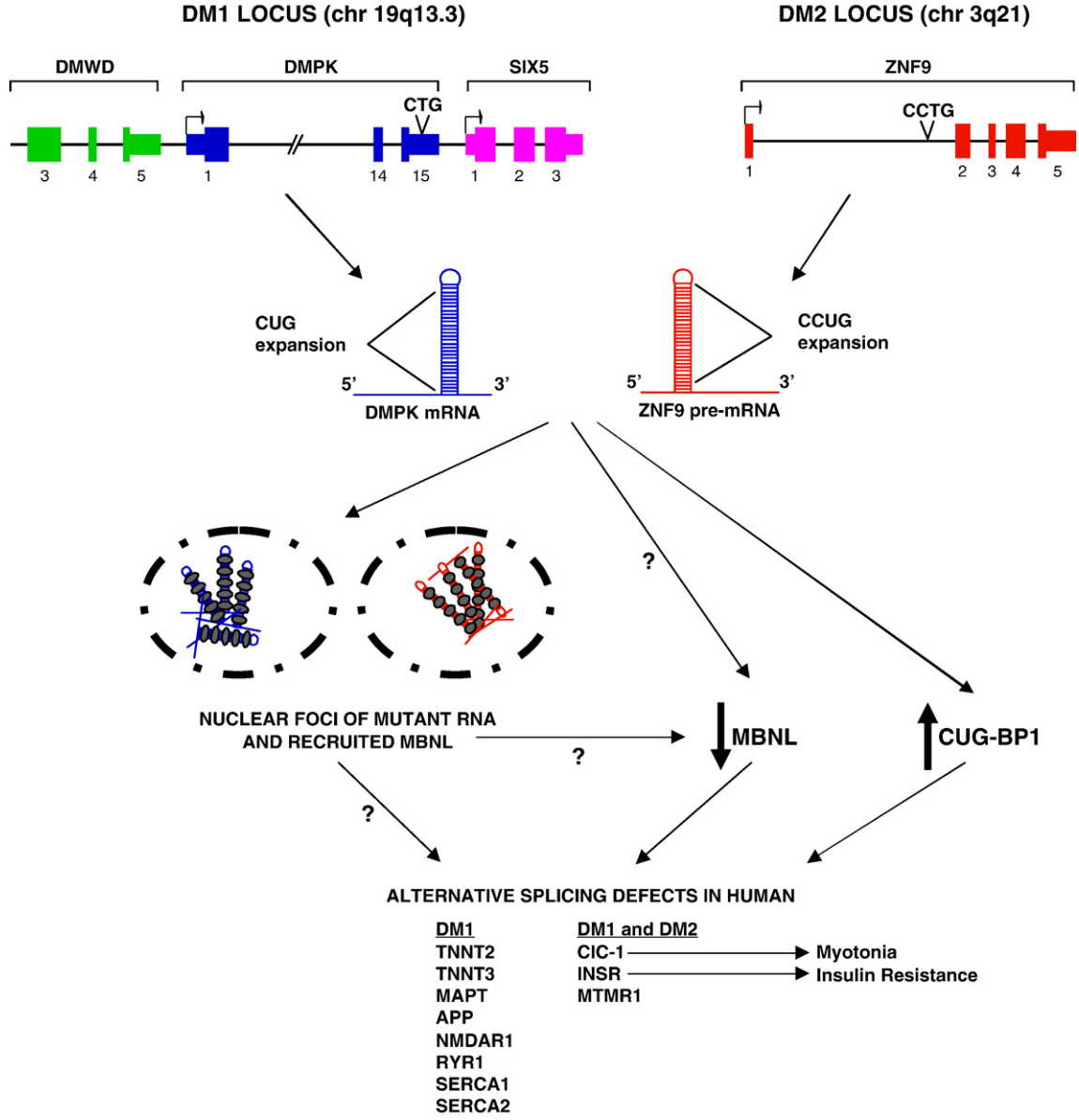

Fig. 2. RNA toxic gain-of-function model for myotonic dystrophy. Myotonic dystrophy is associated with expansions in either CTG repeats in the 3′UTR of *DMPK* or with CCTG repeats in intron 1 of *ZNF9*. RNA transcripts likely form double-stranded hairpin structures from the extended tracts of CUG or CCUG repeats, to which MBNL proteins prefer to bind [70,115]. MBNL proteins co-localize with the ribonuclear inclusions formed by mutant RNA [91]. It is not clear whether nuclear sequestration of MBNL directly leads to a significant decrease in normal MBNL activity, or whether expanded CUG/CCUG RNA signals an alternate pathway to MBNL functional inhibition [79]. CUG-BP1 levels are increased in DM1 cells [65,67], independent of MBNL protein regulation [76]. Increased CUG-BP activity and/or loss of MBNL function may lead to aberrant gene splicing events associated with DM1 and DM2 as shown. So far, it has been shown that myotonia and insulin resistance can be attributed to splicing defects in chloride channel 1 (ClC-1) and insulin receptor (INSR), respectively, in DM1 and DM2 cells [66,67]. Other identified splicing alterations in DM tissues are as follows: TNNT2, cardiac troponin T [62]; TNNT3, skeletal muscle troponin T [74]; MAPT, microtubule-associated protein tau [108]; APP, amyloid beta precursor protein [108]; NMDAR1, *N*-methyl-D-aspartate receptor 1 [108]; RyR1, ryanodine receptor 1 [116]; SERCA, sarcoplasmic/endoplasmic reticulum Ca2+-ATPase [116]; MTMR1, myotubularin-related 1 gene [77,107].

from the discovery that CUG-BP1 regulates alternative splicing of cardiac troponin T (TNNT2) pre-mRNA by directly binding to CUG-repeat-containing splicing enhancers within the transcript, and that this splicing pattern was disrupted in DM1 cardiac and skeletal muscle tissues [62]. There were inconsistencies in trying to fit CUG-BP1 into the emerging model that the trans-dominant effects of the mutant RNA is mediated by the sequestration and inactivation of RNA binding proteins within the intranuclear inclusions found in DM1 cells. The aberrant splicing of TNNT2 indicated that CUG-BP1 activity was increased in DM1, rather than decreased by its titration away from its normal function. Studies also failed to demonstrate co-localization of CUG-BP1 with the foci of mutant DMPK RNA, bringing to question whether CUG-BP1 plays a secondary, rather than primary role in the RNA pathogenesis of DM1 [63,64]. In the meantime, it became evident that increased CUG-BP1 levels in DM1 myoblasts [65] were involved in the aberrant splicing of additional genes such as ClC-1 and INSR, leading to the clinical symptoms of myotonia and insulin resistance, respectively [66,67]. Moreover, transgenic mice engineered to express 250–300 noncoding CUG repeats in the context of either the human skeletal actin gene (HSA^{LR}) or the human *DMPK* gene developed ribonuclear inclusions, myotonia, myopathy, and ClC-1 splicing defects which emphasized the primary role of the mutant RNA on disease pathogenesis [68,69].

Further investigation into proteins that bind to CUG sequences revealed a family of MBNL (muscleblind-like) proteins that bind specifically to the double-stranded RNA hairpins formed by long stretches of CUG repeats [70]. All three isoforms of MBNL proteins (MBNL1, MBNL2, MBNL3) were recruited to the mutant RNA foci in DM1 cells, presumably diverting them from their normal cellular functions [71]. Since the *Drosophila muscleblind* homolog is critical for terminal differentiation in muscle and photoreceptor cells [72,73], MBNL proteins became attractive candidates for the toxic effects of mutant RNA in the pathology of DM1. This trans-dominant RNA theory was further substantiated by the fact that mice homozygous for Mbnl1 deficiency ($Mbnl1^{\Delta 3/\Delta 3}$) developed myotonia, cataracts and splicing irregularities in ClC-1, TNNT2, and skeletal muscle troponin T (TNNT3) which are phenotypic and molecular changes associated with DM1 [74]. Interestingly, it was shown that the splicing activities of MBNL proteins and CUG-BP1 are antagonistic, with MBNL proteins promoting a switch to adult isoforms and CUG-BP1 inducing retention of embryonic isoforms of genes mis-regulated in DM1 tissues such as INSR and TNNT2 [75]. Although their splicing functions are in opposition to one another, MBNL1/MBNL2 and CUG-BP1 do not directly compete with each other as they require different RNA binding sites on their target pre-mRNAs, and their expression levels are independently regulated [76]. Accordingly, transgenic mice overexpressing CUG-BP1 developed signs of muscle defects and abnormal splicing of ClC-1 and TNNT2 similar to $Mbnl1^{\Delta 3/\Delta 3}$ mice [77,78]. Cell culture studies using minigenes and siRNA depletion of MBNL1/MBNL2 or CUG-BP1 indicate that focal accumulation of mutant CUG RNA is primarily dependent on MBNL1, but the aberrant splicing of TNNT2 and INSR genes require CUG-BP1 binding sites [62,67,75]. In addition, nuclear aggregation of MBNL1 with expanded CAG repeats instead of CUG repeats does not correlate with splicing irregularities [79], bringing to question the extent of involvement of parallel processes mediated by MBNL sequestration and CUG-BP1 elevation in DM1 pathogenesis. Additional research will be needed to clarify the exact mechanism by which MBNL1 is functionally inhibited by the CUG repeats, and apparently not by the CAG repeats, and the mechanism of increasing CUG-BP1 expression and activity in DM1.

In addition to the major role of the CUG repeat containing RNA, the chromatin landscape surrounding the pathological repeat motif may contribute to the disease by affecting the expression of neighboring genes at the DM1 locus. CTG repeats were shown to be strong nucleosome positioning elements [80,81], and the expansion of the CTG repeats at the DM1 locus resulted in the occlusion of an adjacent DNase hypersensitive site, signifying a fundamental change in chromatin structure that extends beyond the repetitive sequence motif upon repeat expansion [82]. DMPK is located in a gene-rich region, surrounded by genes less than a kilobase away on either side such as DMWD (dystrophia myotonica-containing WD repeat motif) upstream of its 5′UTR, and SIX5 (sine oculis homeobox homolog 5, formerly DMAHP) immediately downstream of its 3′UTR. Other genes such as RSHL1 (radial spoke head-like) and SYMPLEKIN are all included in a 120 kb region that is flanked by nuclear matrix attachment regions (MARs) that place DMPK and regional genes in the same chromatin fiber loop [83]. The loss of a DNase hypersensitive site located between the CTG repeats in DMPK's 3′UTR and SIX5's promoter on the mutant DMPK allele suggested that this normally 'open' chromatin region becomes 'closed' when the triplet repeats expand, making it less accessible to DNA binding factors for gene regulation. Reporter assays and allele-specific RT-PCRs demonstrated that this normally nuclease-sensitive region contains an enhancer to SIX5, called the hypersensitive site-enhancer (HSE), and that the loss of the HSE associated by the altered chromatin structure caused by the expanded repeat results in suppression of SIX5 from the expanded DMPK allele in human DM1 cells [84,85]. To test that loss of SIX5 expression contributes to the DM1 disease phenotype, SIX5-deficient mice were created by two groups. With increasing severity, heterozygous and homozygous mice for SIX5 deletion developed premature cataracts [86,87]. In a similar dose-dependent manner, loss of SIX5 also resulted in cardiac conduction defects and testicular atrophy [88,89]. With additional evidence that DMWD expression from the mutant allele is also decreased in human DM1 cells [90], it became plausible to suspect that some of the features associated with DM1 might be instigated by repeat expansion-induced chromatin condensation and suppression of local DM1 genes.

In the midst of accumulating evidence for the proposed DMPK haploinsufficiency, trans-dominant RNA, and regional chromatin condensation models to varying degrees, the existence of another DM2 locus associated with the disorder promised to impart more insight into its molecular pathology,

and perhaps provide more validity to one theory over the others. When DM2 was identified as a CCTG expansion in intron 1 of *ZNF9* associated with nuclear inclusions composed of mutant ZNF9 transcripts and MBNL proteins, it reinforced the primacy of the RNA toxic gain-of-function hypothesis [31,91]. Furthermore, alternatively spliced isoforms of ClC-1 and INSR found in DM1 cells were also found in DM2 tissues, implying that similar pathogenic mechanisms were initiated by expanded CUG or CCUG RNA repeats [92,93]. However, an important difference between DM1 and DM2 that might not be explained solely by the toxicity of repetitive elements in RNA, is that only the DM1 locus presents a congenital form with symptoms of mental retardation and hypotonia not shared by DM2. And rather than progressive muscle degeneration, congenital DM1 exhibits muscle immaturity akin to cell culture studies demonstrating inhibition of myogenesis by overexpression of the 3′UTR region of DMPK [94]. This suggests that congenital DM1 might be caused by factors associated with the DM1 locus that add additional pathological mechanisms to those mediated by the CUG containing RNA through MBNL proteins and CUG-BP1 in adult-onset DM1 and DM2.

1.5. DM1 locus and associated congenital DM1

Repetitive elements exist throughout the human genome and are susceptible to epigenetic processes such as bidirectional transcription, histone modification and DNA methylation [95–98]. Congenital DM1 provides evidence for all of the above alterations at the DM1 locus that may have important implications for the unique developmental and clinical manifestation of this disease and perhaps other repeat expansion disorders. Cell culture studies in C2C12 myoblasts demonstrated that stable expression of mutant CUG_{100} RNA generates foci, but does not inhibit myoblast differentiation in the same manner as expressing DMPK's 3′UTR with expanded repeats [99]. Cis-elements adjacent to the repeat domain in DMPK's 3′UTR were thus found necessary for impairing muscle differentiation in C2C12 cells [94,100]. Transgenic mice designed to overexpress the human DMPK 3′UTR with a wildtype number of repeats demonstrated myogenic defects, including reduced fusion potential in primary myoblasts that resembled the delayed muscle development profile of congenital DM1 [101]. In all, influences other than CUG repeats expressed at the RNA level may contribute to congenital DM1 and those factors may involve properties of the DM1 locus.

Prior studies have shown that the CTG repeats in *DMPK* are flanked by CTCF sites [83] that can function as insulators to the effects of nearby enhancers or to the position effects of suppressive chromatin domains [102]. Interestingly, CTCF binding is inhibited by to CpG methylation that occurs in an area encompassing the CTG repeats in congenital DM1 and not adult DM1 [83,103]. Further studies have shown that CTCF binding is indeed absent on the expanded, methylated DM1 allele, but present on the wildtype, non-methylated allele in a congenital DM1 cell line [104]. A model for heterochromatin formation at repetitive DNA sequences implicates dsRNA in triggering an RNAi pathway that produces siRNAs, recruits histone methyltransferases, heterochromatin protein 1 (HP1) and DNA methyltransferases [105]. In comparing the wildtype and expanded alleles in congenital DM1, it was shown that bidirectional transcription extends across the CTG repeats and is converted to siRNA-sized fragments on both alleles, but the antisense transcription is limited by the presence of CTCF on the wildtype allele. On the wild-type allele, this was associated with heterochromatin-associated modifications restricted to the region of the repeat, whereas the functional loss of CTCF next to the expanded repeats was correlated with the spread of antisense transcription and heterochromatin-associated histone modifications to the surrounding regions, which might account for the loss of the nearby HSE and reduced *SIX5* expression [104]. Exclusion of CTCF insulator activity on the expanded allele might also expose the DMPK promoter to the enhancer activity of SIX5′s HSE during embryogenesis when it is most active [86], potentially resulting in elevated levels of expanded DMPK RNA. As shown in cell culture and transgenic mouse studies, increased expression of DMPK results in signs of arrested muscle development and other DM1-associated pathologies that is aggravated by but not dependent on expanded CUG repeats [101,106]. It is currently unknown whether the DM2 locus shares similar chromatin features as DM1.

HSA^{LR} and $Mbnl1^{\Delta 3/\Delta 3}$ mice connect DM1 and DM2 pathogenesis to the RNA toxic gain-of-function hypothesis but fail to demonstrate the congenital form of this disease. More recently, two groups have shown that overexpressing CUG-BP1 in skeletal muscle of mice leads to neonatal lethality at doses from 4 to 10 fold above endogenous levels [77,78]. Histological analysis of skeletal muscle from the CUG-BP1 transgenic mice also revealed defects in myogenic development that was partly characteristic of congenital DM1. The CUG-BP1 transgenic mice displayed DM1-associated splicing defects for Clcn1, and cardiac troponin T (Tnnt2), in addition to myotubularin-related 1 gene (Mtmr1). Although MTMR1 splicing irregularities have not been tested in adult DM1 and DM2 skeletal muscle, it has been shown that MTMR1 is aberrantly spliced in congenital DM1 muscle cell cultures and tissue samples [107], which suggests that the congenital phenotype of this expansion disorder may also result from mis-regulation in alternative splicing through the antagonistic roles of MBNL proteins and CUG-BP1. Mental retardation distinguishes congenital myotonic dystrophy from adult DM1 and DM2, but there have been no reports of CNS involvement in $Mbnl1^{\Delta 3/\Delta 3}$ mice where Mbnl1 function in brains is eliminated. However, transgenic mice overexpressing human DMPK with expanded repeats does lead to altered tau protein isoforms in neurons [69]. Similarly, studies on adult DM1 brain tissues demonstrated ribonuclear inclusions of mutant DMPK RNA and MBNL proteins, as well as splicing alterations in neuronal pre-mRNAs such as NMDA NR1 receptor (NMDAR1), amyloid beta precursor protein (APP), and microtubule-associated protein tau (MAPT) [108]. It remains to be seen whether congenital DM1 is also associated with comparable ribonuclear inclusions and compromises in alternative splicing in the CNS.

1.6. Conclusions and future therapies

As the molecular intricacies of myotonic dystrophy continue to unfold, it is now clear that unstable expansions of CTG or CCTG repeats from genetically distinct contexts can cause this disease syndrome. There is also no doubt that expression of RNA transcripts containing pathogenic repeat lengths can produce defects in alternative splicing of multiple RNAs, providing a basis for the multisystemic features of DM1 and DM2. Therapies targeted to diminishing the toxic effects of CUG repeats show potential in ameliorating the debilitating aspects of this disease. For example, exploiting the endogenous RNAi pathway has been successful in reducing the nuclear accumulation of expanded RNA foci [109,110], and even restoring CUG-BP1 levels and repairing muscle differentiation and insulin insensitivity defects in DM1 myoblasts [111]. Also, catalyzing the cleavage of DMPK RNA in DM1 myoblasts by a hammerhead ribozyme demonstrates a similar reduction in nuclear foci and rescue of INSR splicing alterations [112]. Finally, a survey of chemical compounds to alleviate the toxic effects of expanded CTG repeats in a neuronal cell culture model shows promise in using flavonoids and DHEA-S by an unknown mechanism [113]. There are other prospective targets for therapy such as MBNL proteins, CUG-BP1, and certain transcription factors that may also be sequestered by the expanded RNA transcripts [114]. It is a lasting tribute to the many researchers who have labored to reveal the molecular mechanisms that cause DM1 and DM2 that rational therapies are being developed that will surely ameliorate or cure these diseases.

Acknowledgements

SJT and DHC were supported by NIH AR4203 and AR050741.

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